

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A process for the production of [[a]] biologically active G-CSF protein selected from the group consisting of G-CSF, GM-CSF, M-CSF, EGF, HAS, DNase, FGF, TNF-alpha, TNF-beta, interferons, and interleukins, comprising:

expressing the G-CSF said protein as a heterologous protein in an expression system comprising a cultivated organism having at least one cell, while regulating the following cultivation parameters: temperature of cultivation, composition of cultivation medium, induction mode, type of fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein the protein is expressed as a substantially correctly folded protein precursor in non-classical inclusion bodies, wherein the protein precursor has an aqueous solubility, in non-classical inclusion bodies, and wherein regulating the cultivation parameters increases the proportion of substantially correctly folded protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in a cell of an organism not cultivated while regulating said parameters;

regulating one or more cultivation parameters selected from the group consisting of temperature of cultivation, composition of cultivation medium, induction mode, principle of performing the fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein regulating the one or more parameters increases the proportion of substantially correctly folded protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in a cell of an organism not cultivated by regulating said parameters;

isolating the non-classical inclusion bodies from the cell of the organism;

optionally, washing the non-classical inclusion bodies;

solubilizing the substantially correctly folded protein precursor from the non-classical inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies with a non-denaturing aqueous solvent having a pH of about 8.0; and

purifying the biologically active protein from the solubilized substantially correctly folded protein precursor and non-denaturing aqueous solvent,

wherein the process for the production of the biologically active G-CSF protein is free from any denaturation and renaturation of the G-CSF protein.

2 – 4. (Cancelled).

5. (Previously Presented) A process for the production of a protein according to claim 1, wherein the cultivated organism is selected from the group consisting of bacteria and yeasts.

6. (Previously Presented) A process for the production of a protein according to claim 5, wherein the cultivated organism is the bacterium *E. coli*.

7. (Previously Presented) A process for the production of a protein according to claim 1, wherein the heterologous protein is accumulated in the inclusion bodies to a proportion of at least about 10%, relative to the total protein mass of a cell of the organism used in the expression system.

8 – 9. (Cancelled).

10. (Previously Presented) A process according to claim 1, wherein the temperature of cultivation ranges from about 20° C to about 30° C.

11. (Cancelled).

12. (Currently Amended) A process according to claim 1, wherein regulating the induction mode comprises selecting an inducer from the group consisting of IPTG, lactose, and NaCl, and combinations thereof.

13. (Previously Presented) A process according to claim 12, wherein the selected inducer is IPTG.

14. (Previously Presented) A process according to claim 13, wherein the concentration of IPTG ranges from about 0.1 mM to about 1 mM.
15. (Previously Presented) A process according to claim 14, wherein the concentration of IPTG is about 0.4 mM.
16. (Previously Presented) A process according to claim 12, wherein the regulation of the induction mode further comprises adding the inducer at the beginning of the fermentation.
17. (Currently Amended) A process according to claim 1, wherein the type of fermentation principle of performing the fermentation is selected from the group consisting of performing of fermentation in a batch mode, performing of fermentation in a fed batch mode, and performing of fermentation in one or more shake flasks, and combinations thereof.
18. (Canceled).
19. (Currently Amended) A process according to claim 1, wherein the composition of the cultivation medium is selected from the group consisting of GYST, GYSP, LYSP, LYST, LBON and GYSPO, and combinations thereof.
20. (Previously Presented) A process according to claim 19, wherein the selected medium is GYST, or GYSP.
21. (Currently Amended) A process according to claim 1, wherein the stress induction agent is selected from the group consisting of ethanol, and propanol, and combinations thereof.
22. (Canceled).
23. (Currently Amended) A process according to claim 1, wherein the step of washing comprises contacting the inclusion bodies with a solution selected from the group consisting of Tris/HCl buffer, phosphate buffer, acetate buffer, citrate buffer, and water, and combinations thereof.

24. (Previously Presented) A process according to claim 23, wherein the concentration of the selected buffer ranges from about 1 mM to about 10 mM.

25. (Previously Presented) A process according to claim 23, wherein the selected solution is water.

26. (Currently Amended) A process for production of a protein according to claim 1, wherein the non-denaturing aqueous solvent is selected from the group consisting of aqueous solutions of: urea ranging in concentration from about 1M to about 2M, N-lauroyl sarcosine ranging in concentration from about 0.05% to about 0.25% mass per volume, betain, sarcosine, carbamoyl sarcosine, taurine, DMSO, non-detergent sulfobetains, and a buffer in a high, solubilising concentration, and combinations thereof, said buffer being selected from the group consisting of HEPES, HEPPS, MES, and ACES, and combinations thereof.

27-37. (Canceled).

38. (Previously Presented) The process of claim 26, wherein the non-denaturing aqueous solvent comprises a relatively low concentration of N-lauroyl sarcosine in water, in order to avoid denaturing conditions.

39. (Previously Presented) The process of claim 38, wherein the concentration of N-lauroyl sarcosine further ranges from about 0.1% to about 0.25% mass per volume.

40. (Previously Presented) The process of claim [[4]] 1, wherein the specific activity of the G-CSF is at least  $1 \times 10^7$  IU/mg.

41. (Previously Presented) The process of claim 1, wherein the amount of protein expressed is at least about 20% by mass of the total mass of proteins produced by the host cell.

42. (Previously Presented) The process of claim 1, wherein the amount of protein expressed is at least about 30% by mass of the total mass of proteins produced by the host cell.

43. (New) A process for the production of biologically active G-CSF, comprising:

expressing the G-CSF as a heterologous protein in an expression system comprising at least one *E. coli* bacterium, while regulating the following cultivation parameters: temperature of cultivation, composition of cultivation medium, induction mode, type of fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein the protein is expressed as a substantially correctly folded protein precursor in non-classical inclusion bodies, wherein the protein precursor has an aqueous solubility, and wherein regulating the cultivation parameters increases the proportion of substantially correctly folded protein precursor present in the non-classical inclusion bodies in the cell, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in a cell of a bacterium not cultivated while regulating said parameters;

isolating the non-classical inclusion bodies from the cell of the organism;

optionally, washing the non-classical inclusion bodies;

solubilizing the substantially correctly folded protein precursor from the non-classical inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies with a non-denaturing aqueous solvent having a pH of about 8.0; and

purifying the biologically active protein from the solubilized substantially correctly folded protein precursor and non-denaturing aqueous solvent,

wherein the process for the production of the biologically active G-CSF is free from any denaturation and renaturation of the G-CSF

and wherein the temperature of cultivation is from about 20° C to about 30° C, the type of fermentation is fed-batch, and the induction mode is regulated using IPTG as an inducer.

44. (New) A process for the production of biologically active G-CSF according to claim 43, wherein the G-CSF is accumulated in the inclusion bodies to a proportion of at least about 30%, relative to the total protein mass of a cell of the *E. coli* used in the expression system.

45. (New) A process according to claim 43, wherein the concentration of IPTG ranges from about 0.1 mM to about 1 mM.

46. (New) A process according to claim 45, wherein the concentration of IPTG is about 0.4 mM.
47. (New) A process according to claim 43, wherein the composition of the cultivation medium is selected from the group consisting of GYST, GYSP, LYSP, LYST, LBON and GYSPO, and combinations thereof.
48. (New) A process according to claim 43, wherein the stress induction agent is selected from the group consisting of ethanol, propanol, and combinations thereof.
49. (New) A process according to claim 1, wherein the step of washing comprises contacting the inclusion bodies with a solution selected from the group consisting of Tris/HCl buffer, phosphate buffer, acetate buffer, citrate buffer, water, and combinations thereof.
50. (New) A process according to claim 49, wherein the concentration of the selected buffer ranges from about 1 mM to about 10 mM.
51. (New) A process according to claim 49, wherein the selected solution is water.
52. (New) A process for production of a protein according to claim 43, wherein the non-denaturing aqueous solvent is selected from the group consisting of aqueous solutions of: urea ranging in concentration from about 1M to about 2M, N-lauroyl sarcosine ranging in concentration from about 0.05% to about 0.25% mass per volume, betain, sarcosine, carbamoyl sarcosine, taurine, DMSO, non-detergent sulfobetains, a buffer in a high, solubilising concentration, and combinations thereof, said buffer being selected from the group consisting of HEPES, HEPPS, MES, and ACES, and combinations thereof.
53. (New) The process of claim 43, wherein the non-denaturing aqueous solvent comprises a relatively low concentration of N-lauroyl sarcosine in water, in order to avoid denaturing conditions.

54. (New) The process of claim 53, wherein the concentration of N-lauroyl sarcosine further ranges from about 0.1% to about 0.25% mass per volume.

55. (New) The process of claim 43, wherein the specific activity of the G-CSF is at least  $1 \times 10^7$  IU/mg.